United States Environmental Protection Agency

Revisiting and updating chemical groupings with new approach methodologies

US EPA in collaboration with Health Canada, Environment Climate Change Canada

APCRA-5

August 26 – 27, 2020

Office of Research and Development
Center for Computational Toxicology and Exposure

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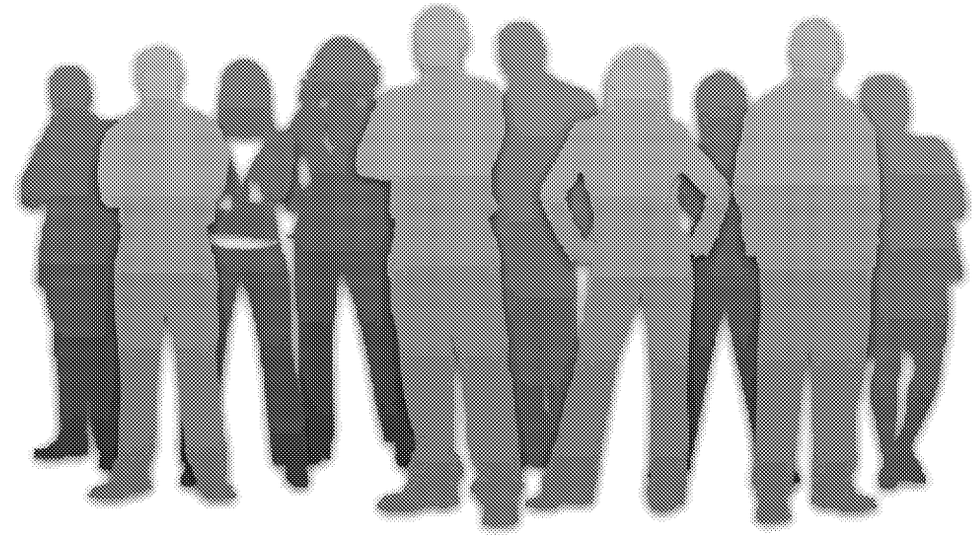

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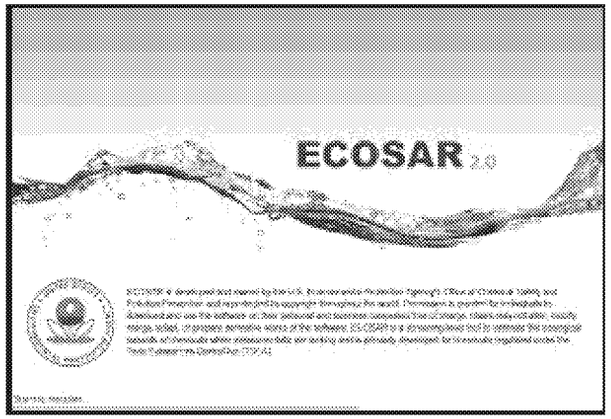
Overview

A chemical category is a group of chemicals whose physicochemical and human health and/or ecotoxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern, usually as a result of structural similarity. - OECD

Applications of chemical categorization include first tier assessment efforts and read across from structurally similar analogs:

- Toxic Substances Control Act (TSCA) New Chemical Program Chemical Categories (NCC; US EPA)
- ECOSAR (focus of presented work)

US EPA ECOSAR chemical classifications



- Class-based SAR to predict aquatic toxicity
- Classification scheme identifies excess toxicity
- Estimates **acute** and **chronic toxicity** based on accumulated data and past decisional precedents

Acute Effects:	Chronic Effects:
Fish 96-hr LC_{50}	Fish ChV
Daphnid 48-hr EC_{50}	Daphnid ChV
Algae 72/96-hr EC_{50}	Algae ChV

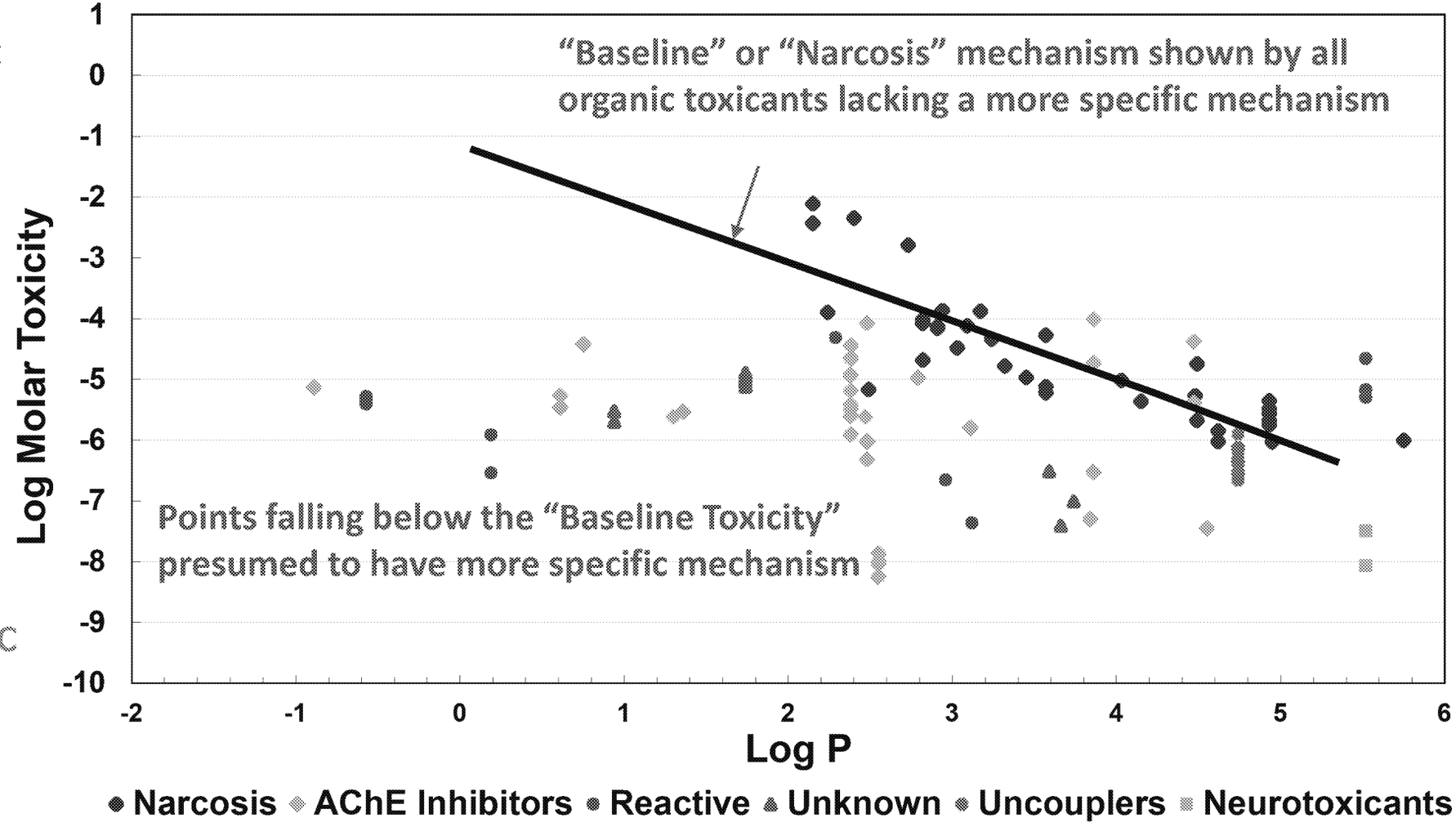
- Profiler in OECD QSAR Toolbox

Narcosis vs. specific-acting toxicity MOA

Less Toxic

Regulators (ECCC) consider MOA information to determine the size of assessment factors

More Toxic



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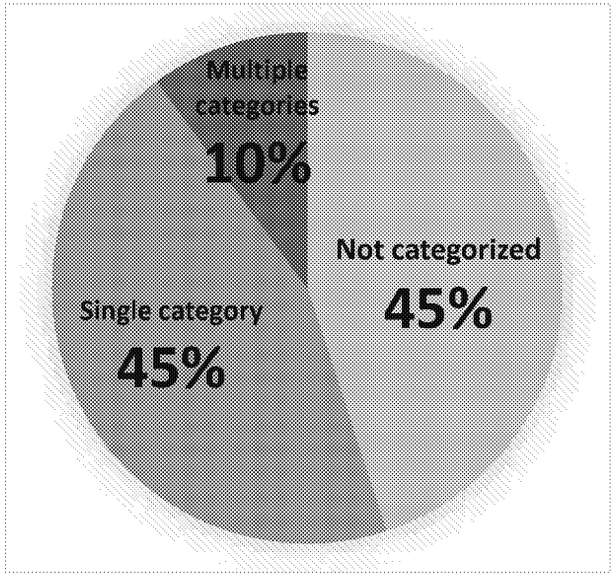
Potential approach for updating chemical categories



- Almost half of all New Chemical inventories across regulatory jurisdictions cannot be categorized using NCC or ECOSAR
- Some fall into multiple categories

How do we update?

- Incorporate New Approach Methodologies (NAMs) – *i.e.*, ToxCast and Tox21 biological activity information
- Apply cheminformatic approaches

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ToxPrint (TxP) model development

General approach

Training set chemicals

- Well-defined MOA (narcosis vs. specific-acting)
- NAM data *in vitro* toxicity data
- *in vivo* toxicity data
- Representative of chemicals of interest for prediction

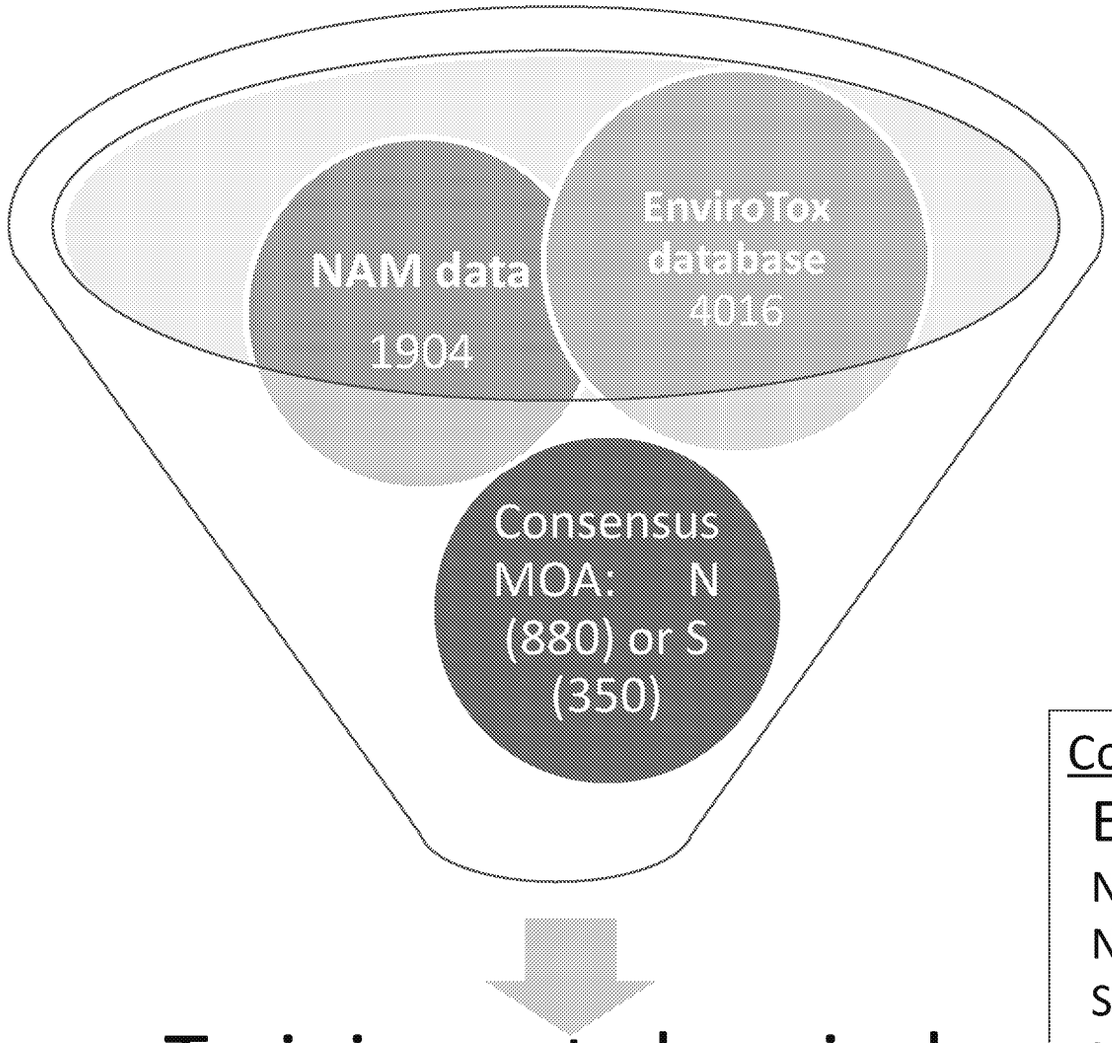
Characterize training set

- 1. ECOSAR classes
- 2. NCC
- 3. Chemotype fingerprints (ToxPrints)

Model

- NAM data, chemotypes and combination of both
- Evaluate different machine learning algorithms

EnviroTox training set chemicals



1. Chemicals with *in vivo* eco-data – from the EnviroTox[1] database – 4016

2. Sub-selection for chemicals with NAM data (ToxCast and Tox21) - 1904

3. MOA predictions based on 4 publicly-available classification models
   - VERHAAR, ASTER, OASIS, TEST
   - Each predicts Narcotic, Specific-Acting or Unclassified

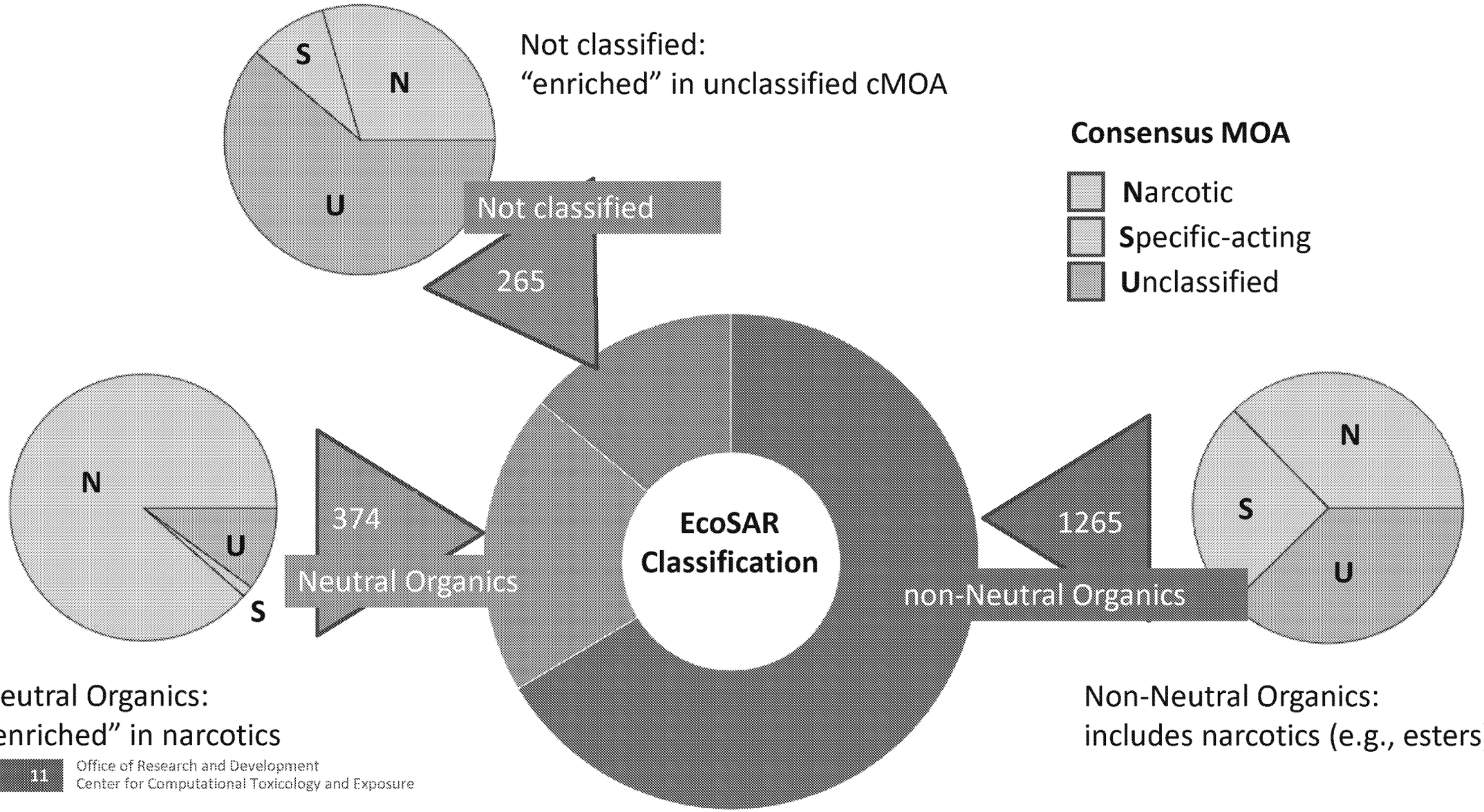
Consensus MOA (cMOA) with confidence scores[2]

Examples:	Results:
NNNN = N, score =3	880 Narcotic
NNSN = N, score= 2	350 Specific-acting
SUSS = S, score= 2	674 Unclassified
NUNS = U, score = 0	

[1]Health and Environmental Sciences Institute (HESI). 2019. EnviroTox Database & Tools. Version 1.1.0 Available: http://www.envirotoxdatabase.org/

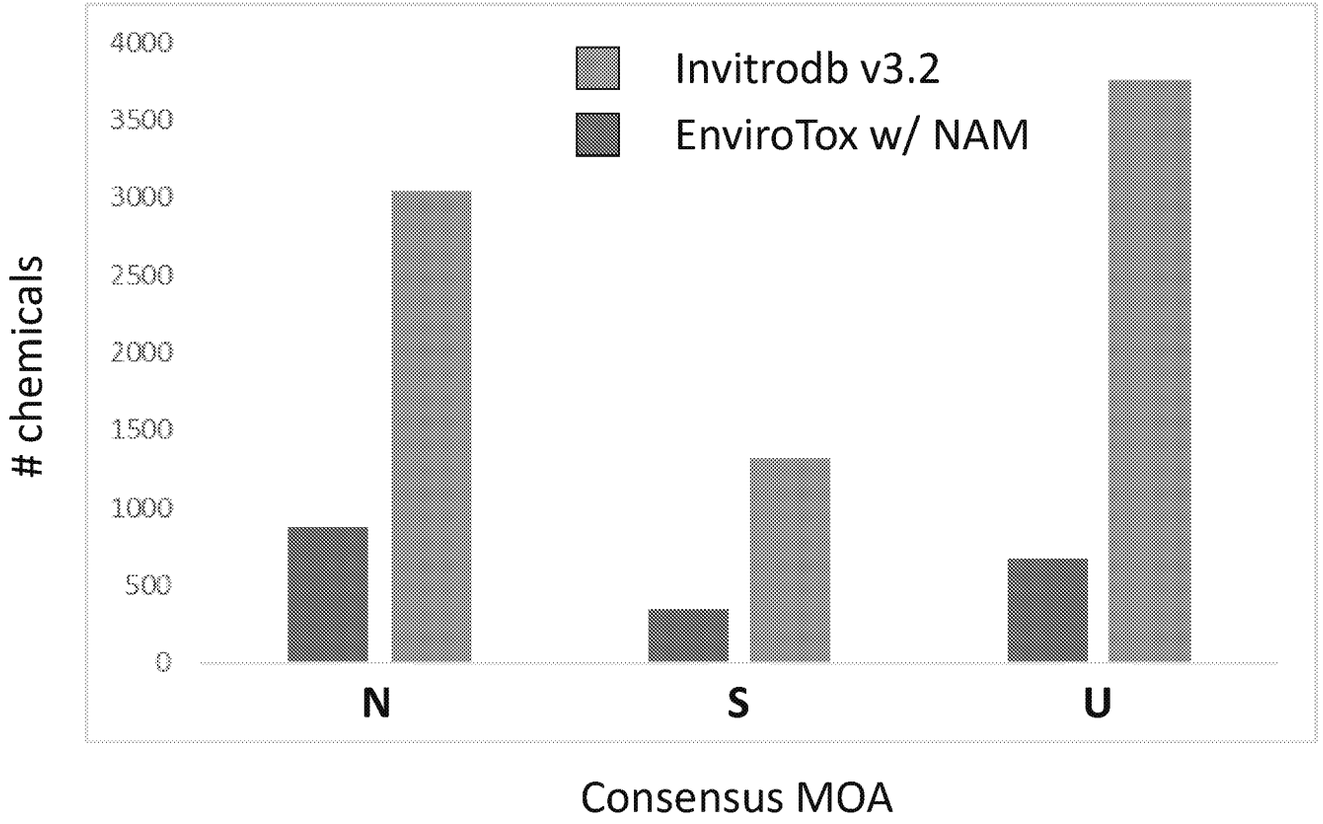
[2] Kienzler et al.. Environ Toxicol and Chem. 2019, 38(10) 2294-2304

Characterize EnviroTox training set chemicals: ECOSAR classes



Expanding the Envirotox chemical space

- Additional 6215 chemicals with NAM data (invitrodb v3.2)
- Applied the same consensus MOA methodology



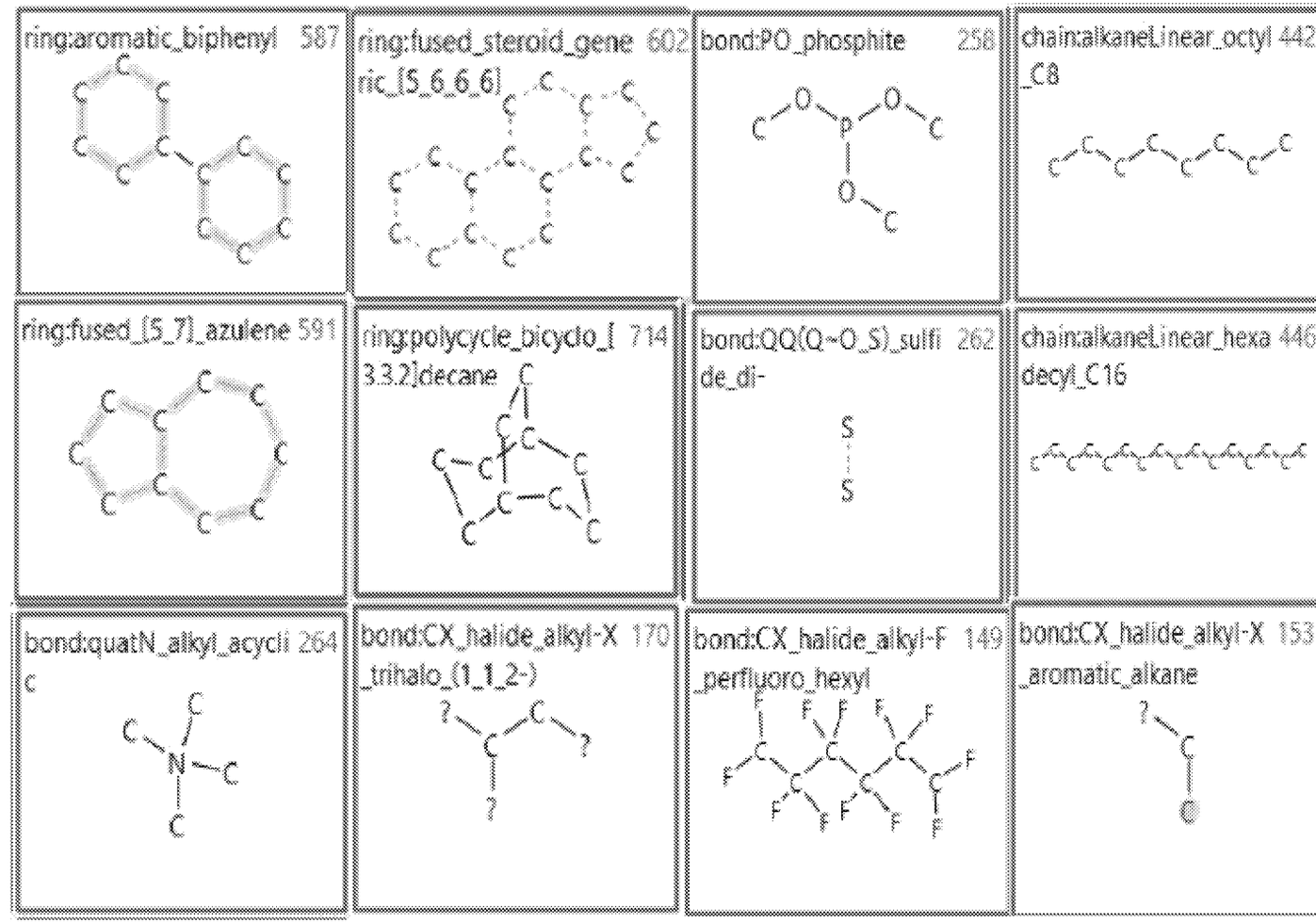
- Increased chemical coverage across all classes, specifically in the unclassified cMOAs relative to N/S classes

Characterize training set chemicals: ToxPrints

- Pull in chemotype information for our chemicals via ToxPrints (TxPs)
  - Publicly available tool
  - EPA Comptox Chemicals Dashboard

ToxPrints:

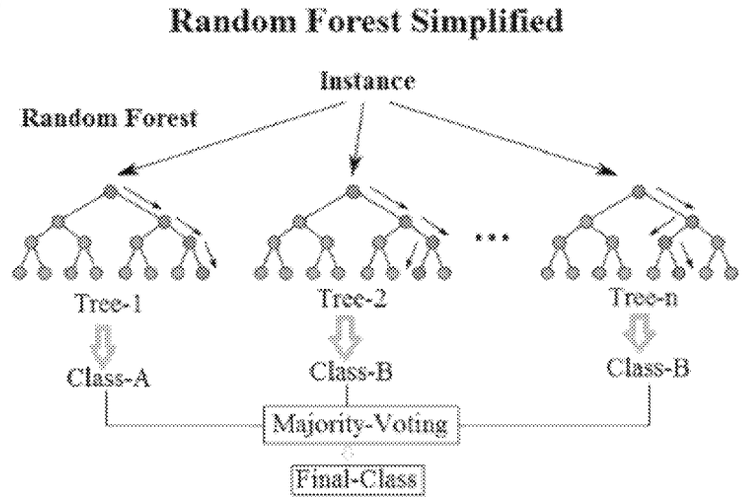
- ✓ *729 chemical features*
- ✓ *Chemically interpretable*
- ✓ *Coverage of diverse chemistry*
- ✓ *Hierarchical: Includes scaffolds, functional groups, chains, rings, bonding patterns, atom-types*



Yang et al. J. Chem. Inf. Model. 2015. Richard et al., Chem. Res. Toxicol. 2016, 29(8) 1225 – 1251; Strickland et al., Arch Toxicol. 2018 92(1) 487 – 500; Wang et al., Environment International 2019, 126 377 – 386

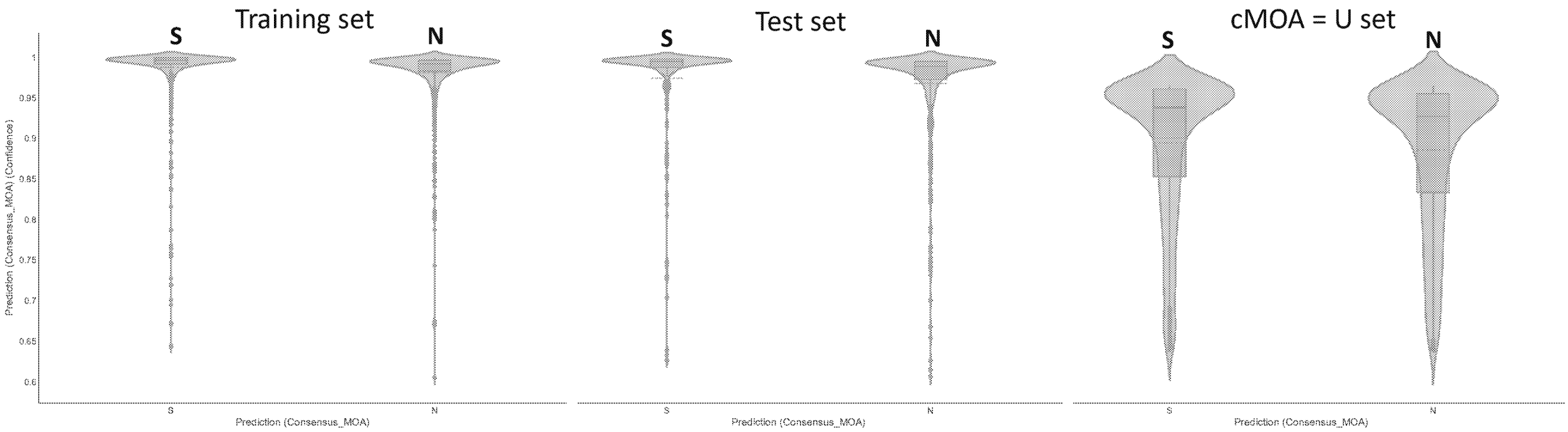
TxP model details

- Random Forest (Boosted Gradient Method) provided the best model results:
  - Split data into 80% training and 20% hold out (test) sets
  - Hyperparameter tuning with 5-fold cross validation, square-root sampling, etc.
  - Training set: “balanced” down-sampled subset (2104 chemicals w/ a cMOA = N or S)
  - High accuracy in both training and test sets (training = 99.7%; test = 95.8%)
  - Total Accuracy on all N + S data set = 97.6% (4356 cMOA = N or S)
    - Across all N + S chemicals -> 105 chemicals misclassified:
      - 24 F_{pos}{predicted S}
      - 81 F_{neg}{predicted N}



https://medium.com/@williamkoehrsen/random-forest-simple-explanation-377895a60d2d

Distribution of prediction confidence [0,1] by (N,S) class



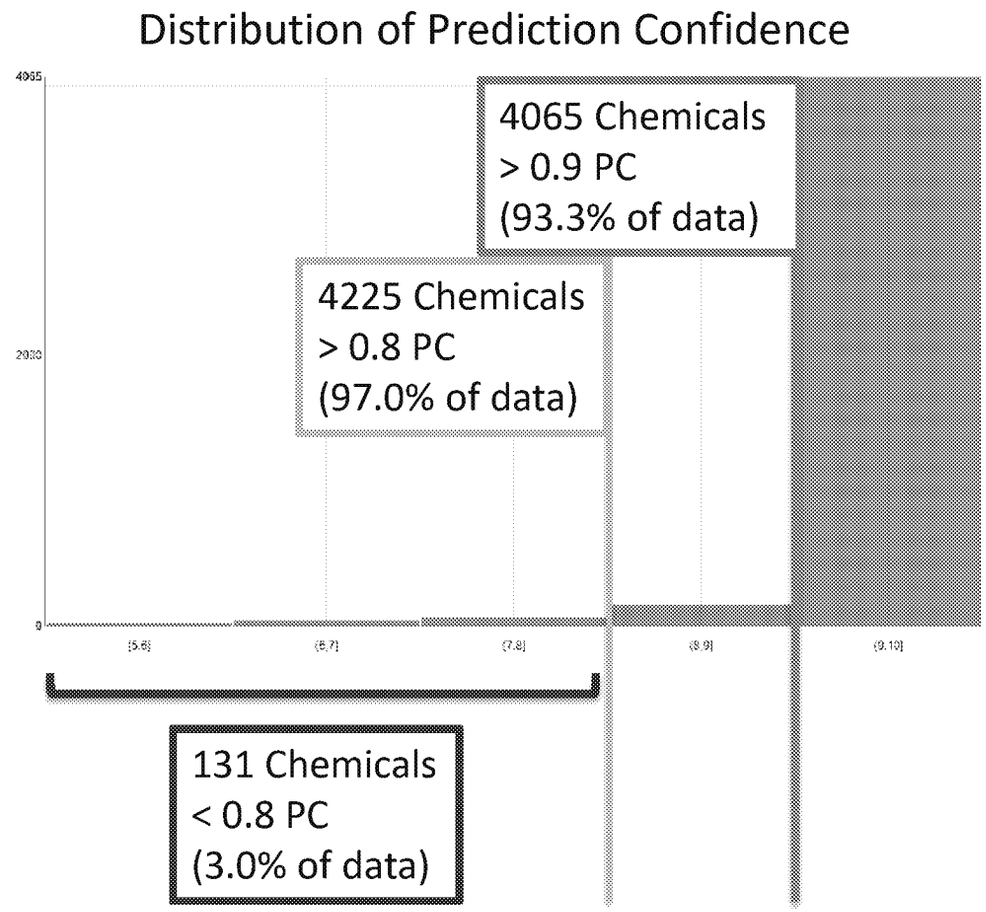
Training Set
Median: 0.999, 0.993
Mean: 0.988, 0.982

Test Set
Median: 0.996, 0.989
Mean: 0.970, 0.962

Unclassified Set
Median: 0.958, 0.941
Mean: 0.892, 0.877

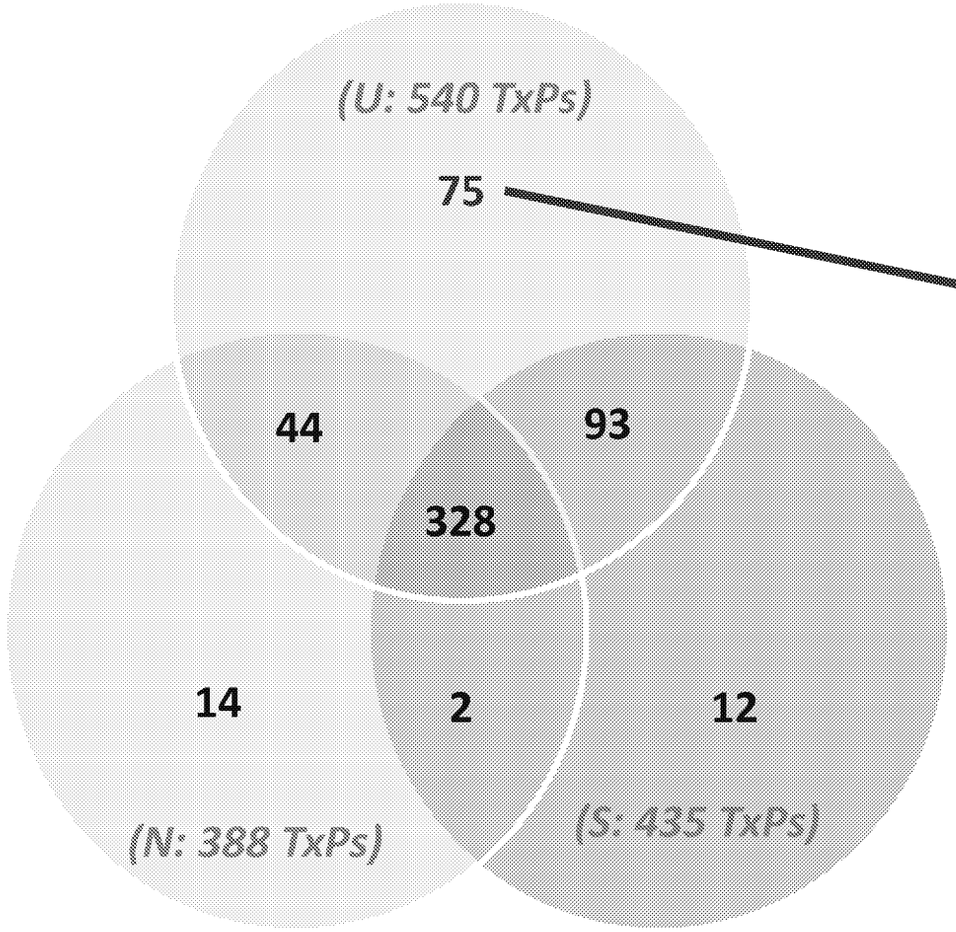
Prediction confidence across the cMOA = N or S

- Distribution of prediction confidence (PC) tends to be > 0.8 for the classified data (cMOA = N or S)
- Model has fewer # misclassifications in S
  - Misclassifications for 93 cMOA confidence = 2, and 12 with 1,3 scores (recall 3>2>1 for confidence)
  - **~46% of the misclassifications can be attributed to the chemicals with PC < 0.8**
  - ~67% of the misclassification can be attributed to chemicals with PC < 0.88

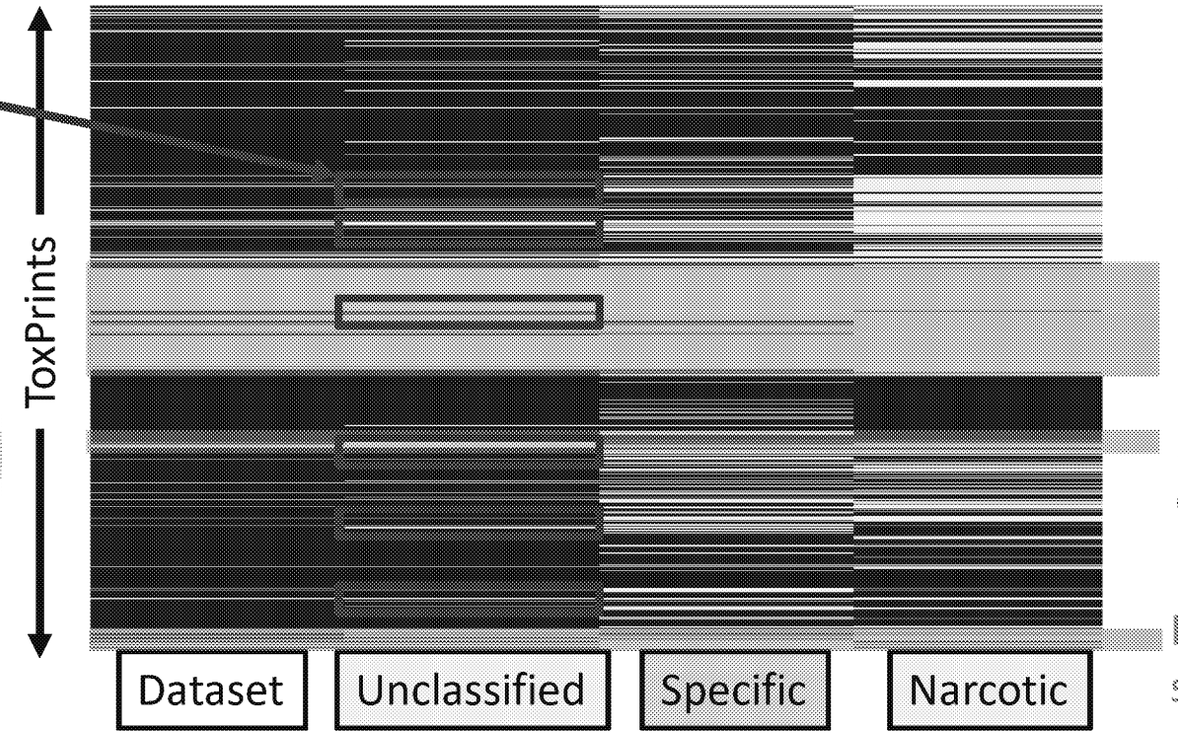


ToxPrint (TxP) domains

Characterization of TxP coverage per consensus MOA class



Heatmap representation of ToxPrints



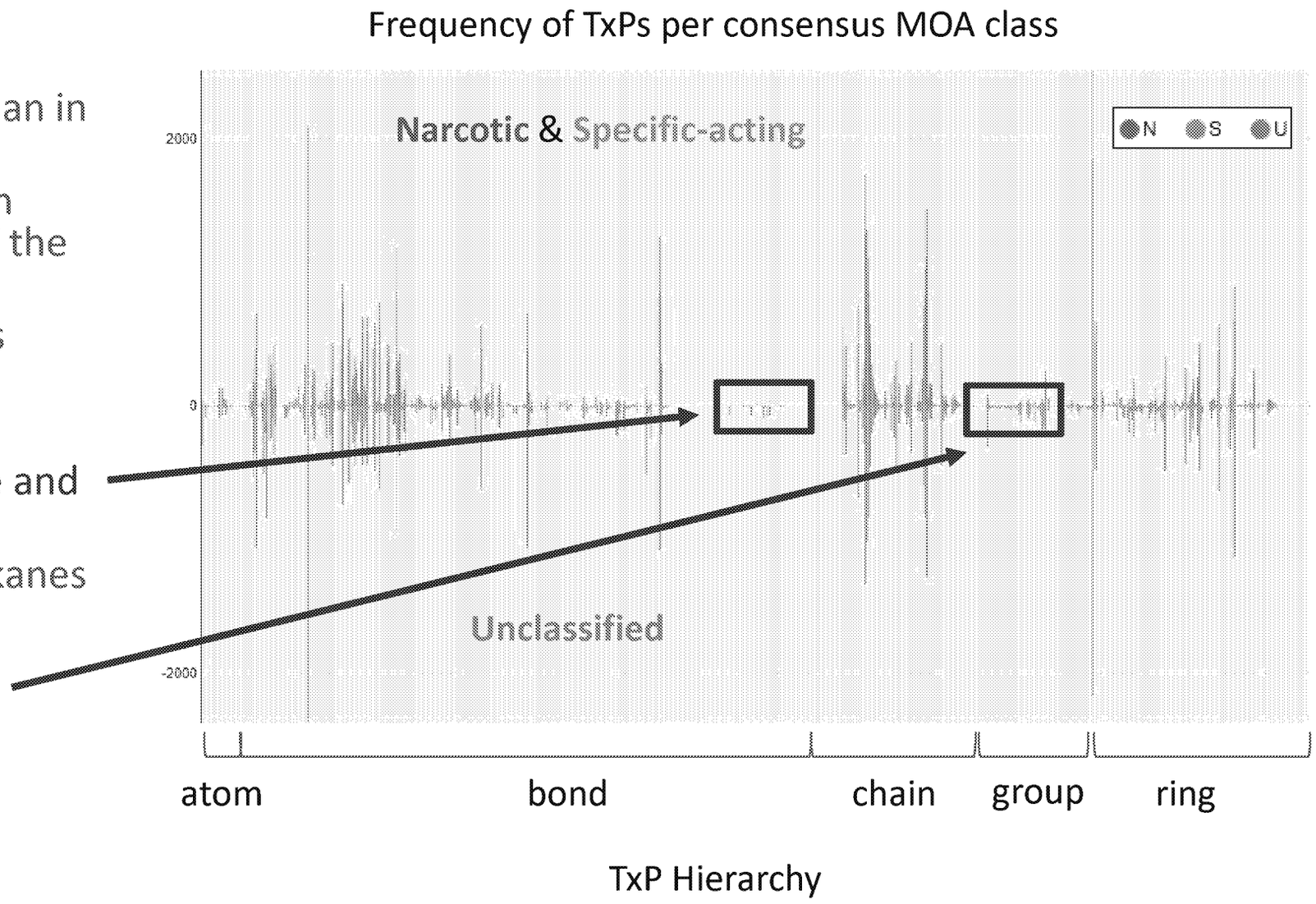
Missing structural Domains across full dataset:

- Metal & metalloid bonds
- Propyleneoxide chains
- Bicyclo ring structures

ToxPrints: Dataset > Unclassified > Specific-acting > Narcotic

Unique TxPs in the unclassified set

- ~7x more unique features in U (than in N or S)
- Could explain the lower prediction confidence in N/S classification of the U set
- Potential for additional categories based on structure:
  - 2 atom TxPs (metal group III)
  - 38 bond TxPs (metalloid: silane and siloxanes…)
  - 8 chain TxPs (ethyleneoxide alkanes C10 – C20)
  - 19 group TxPs (amino acids, polydentate ligands)
  - 8 ring TxPs

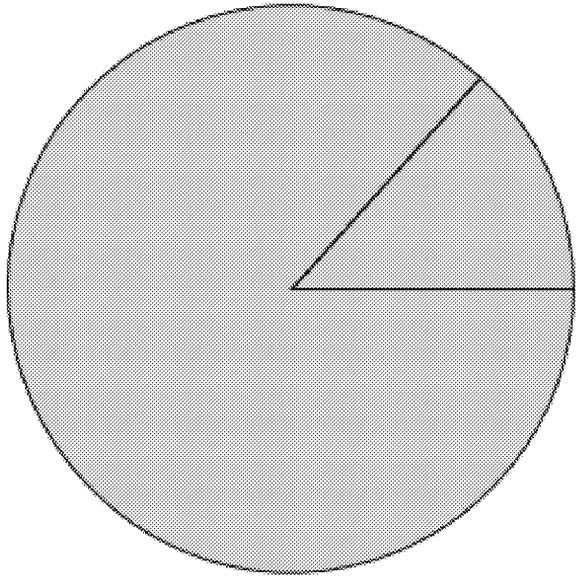


ToxPrint (TxP) model application to Envirotox dataset

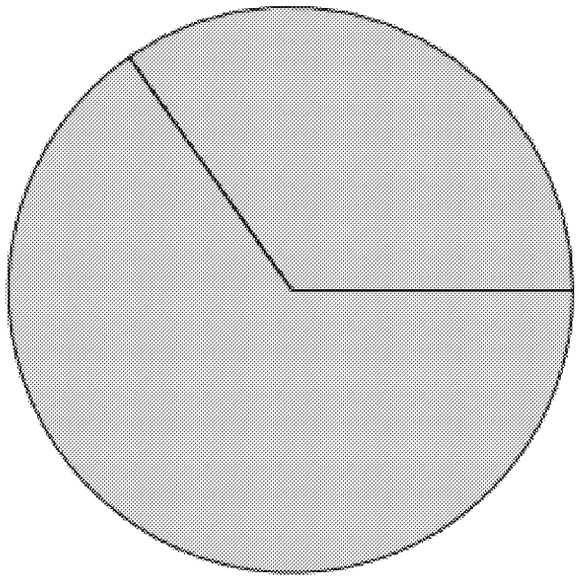
TxP model predicted MOAs of the EnviroTox unclassified set

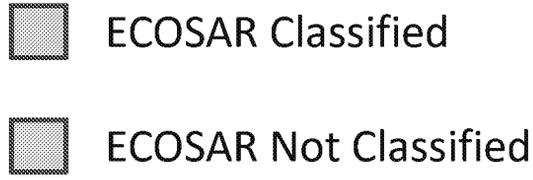
- 674 chemicals in the EnviroTox dataset that had low confidence or ambiguous consensus
- Applied TxP model to the unclassified set and compared predictions to ECOSAR classification
- ***Currently extending this analysis to the additional 3089 unclassified chemicals***

361 predicted as Narcotic



313 predicted as Specific-acting





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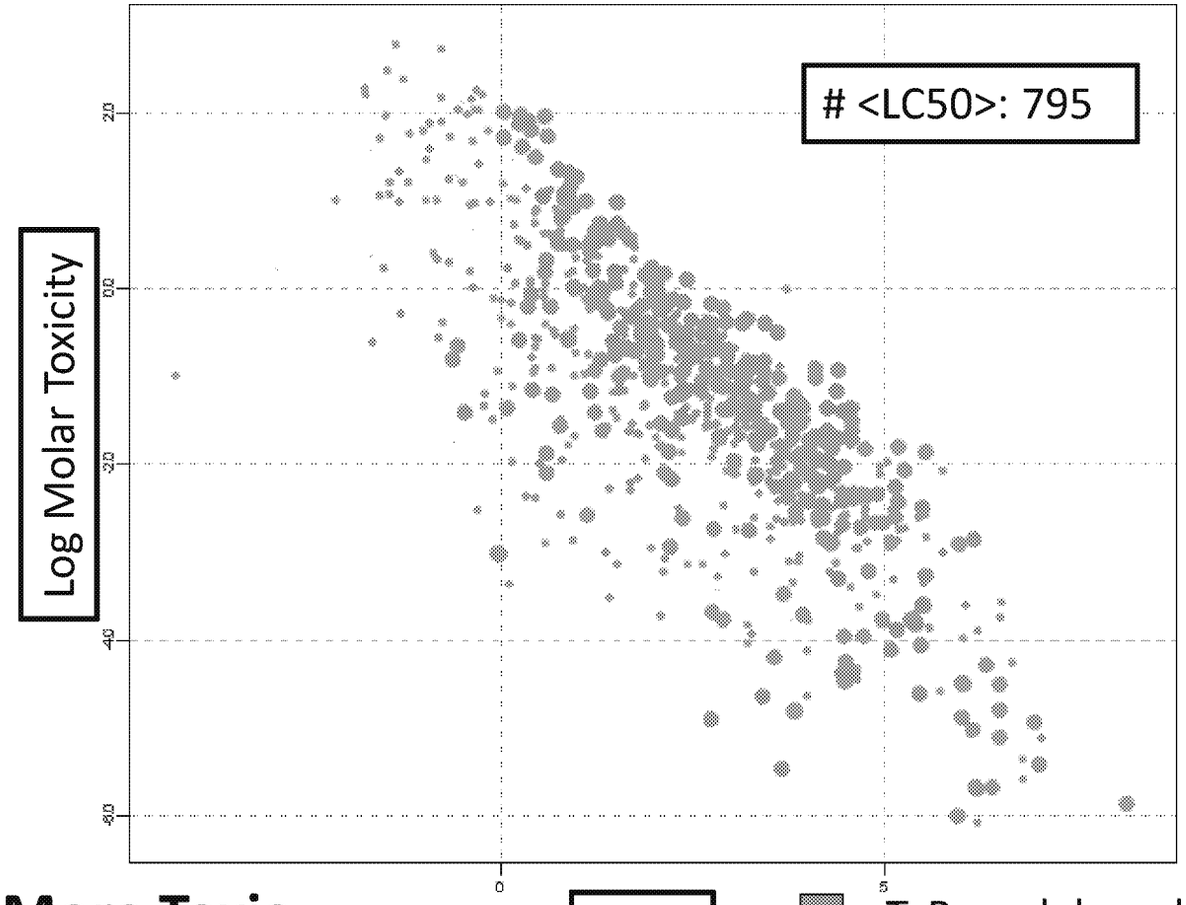
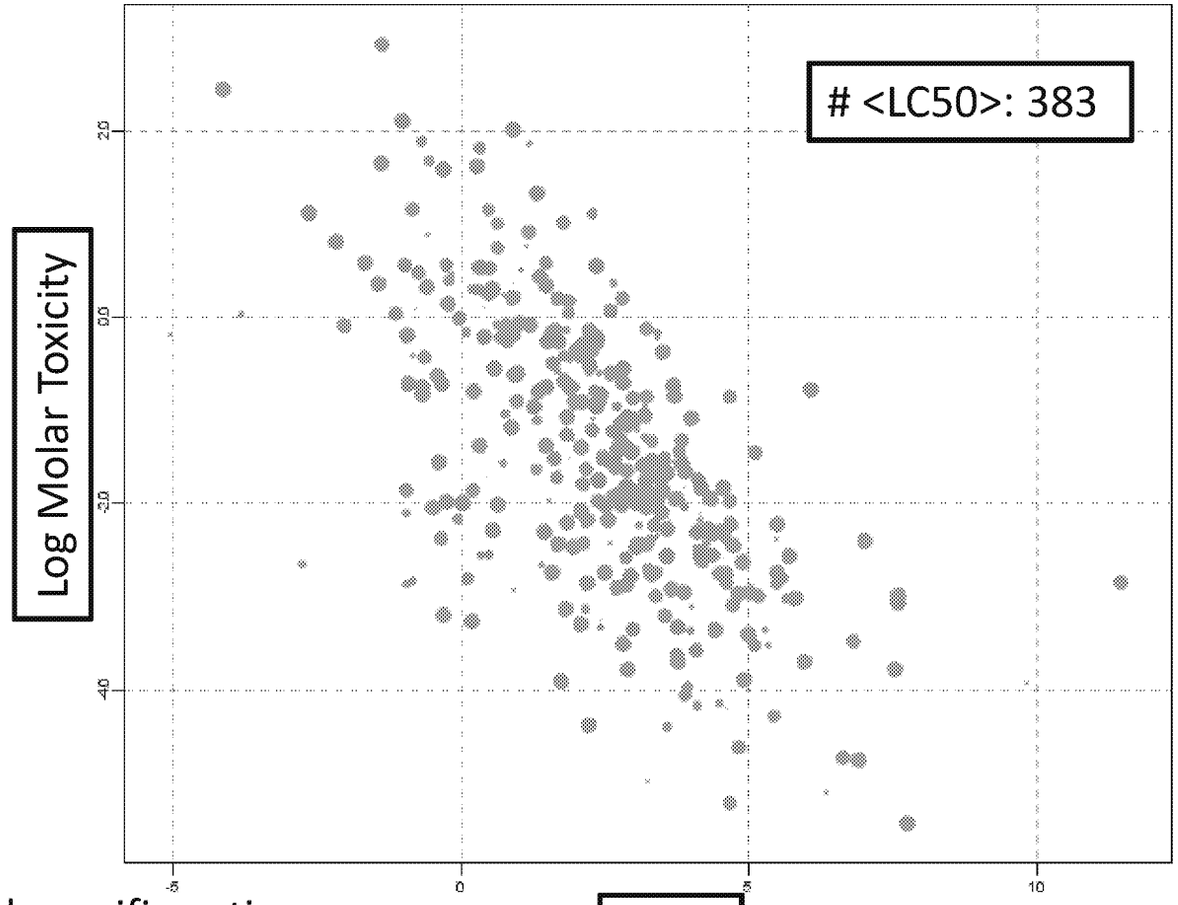
Log molar toxicity, (LC50, 96h, FISH): TxP model predicted MOA (N,S) for cMOA (N,S,U) data

Less Toxic

cMOA classified data (N,S)



cMOA unclassified data (U)



More Toxic

TxP model predicted specific-acting

TxP model predicted narcotic

Size proportional to cMOA confidence score = [0,1,2,3]

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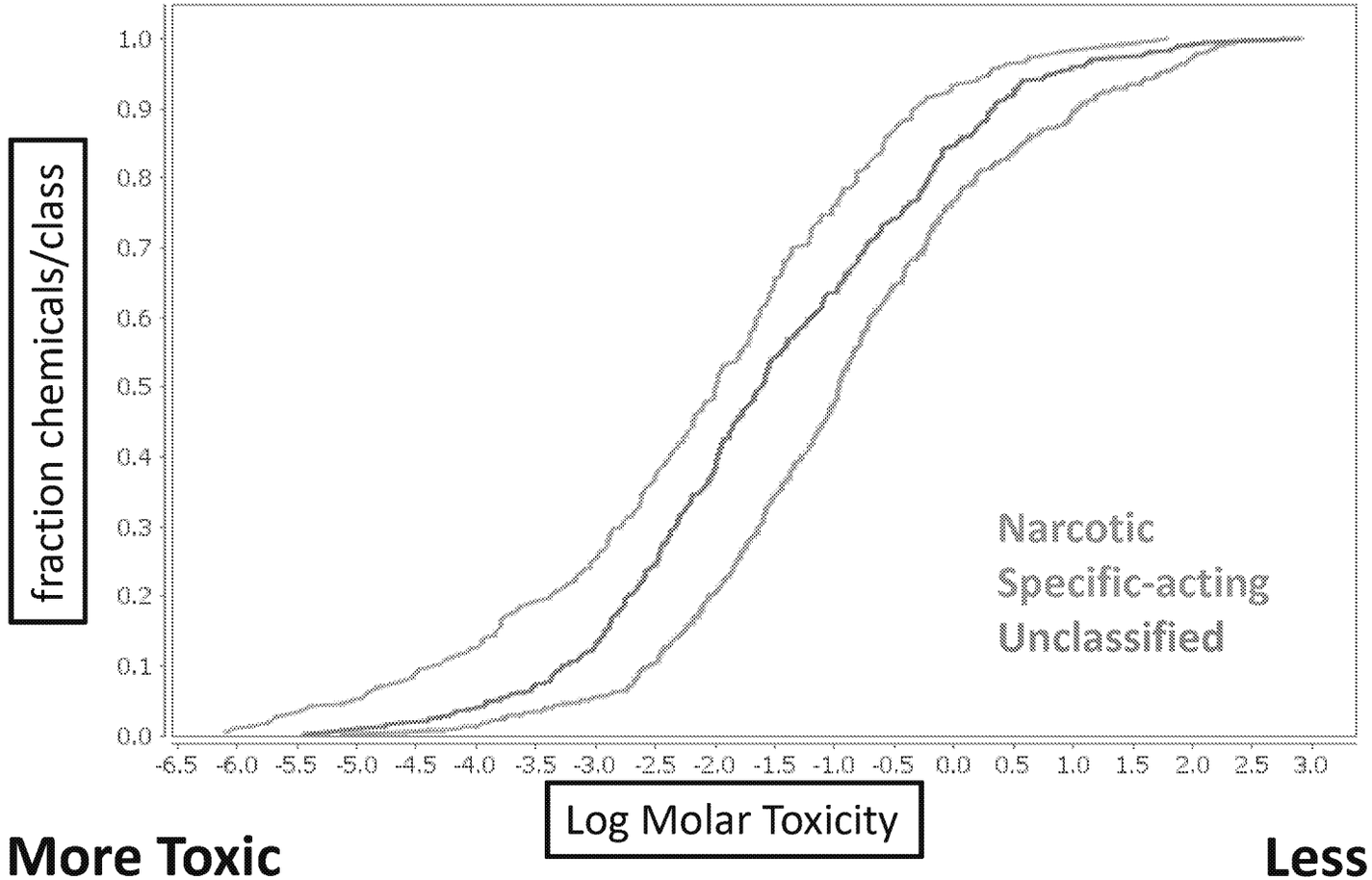
Cumulative distribution function: Log molar toxicity, (LC50, 96h, FISH) for cMOA classes (N,S,U)



- cMOA classification is sufficient to discriminate N,S
- U presents some challenges

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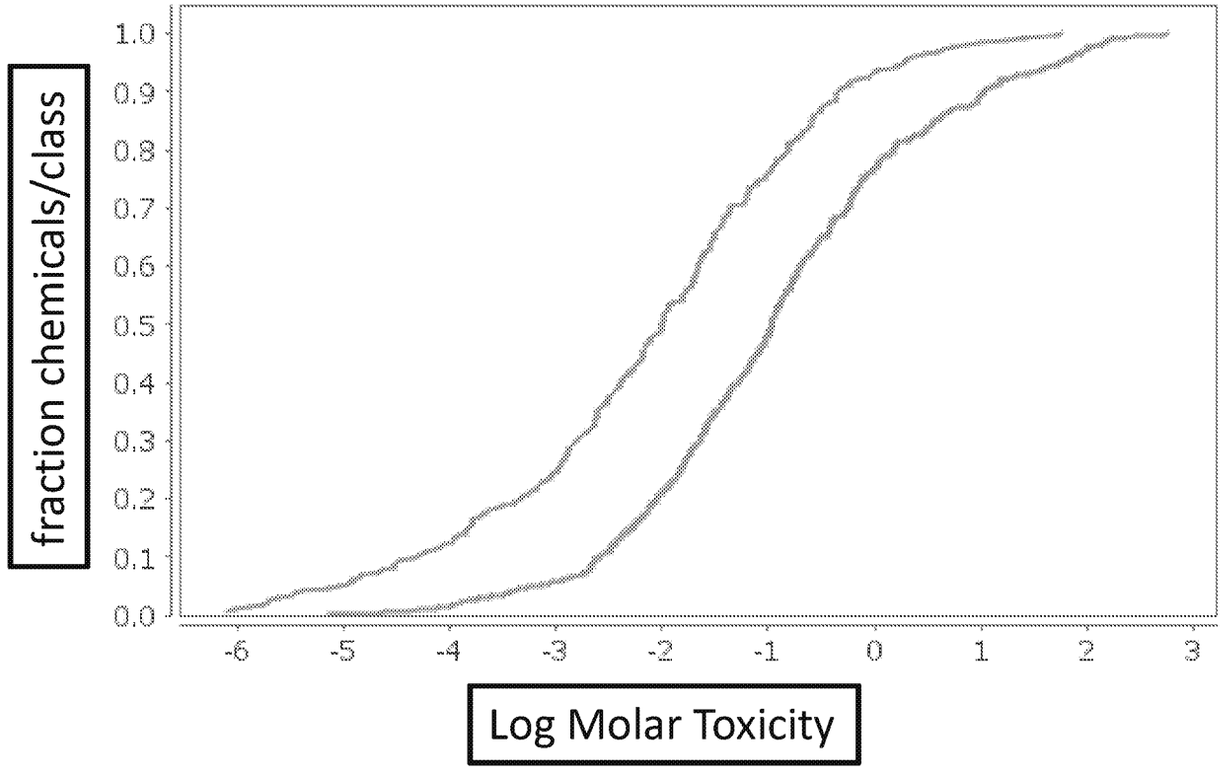
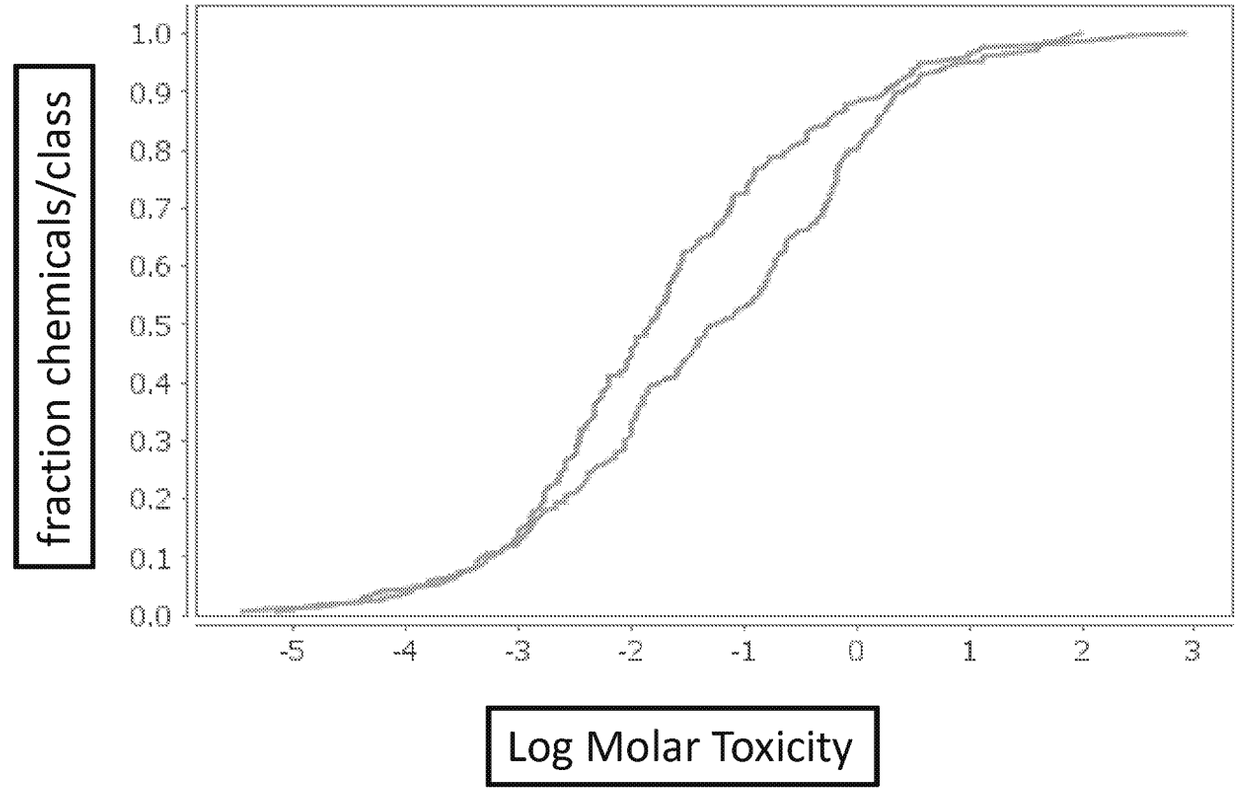
Cumulative Distribution Function: Log molar toxicity, (LC50, 96h, FISH) for TxP model predicted classes (N,S)

cMOA classified data (N,S)



cMOA unclassified data (U)



TxP model predicted specific-acting

TxP model predicted narcotic

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Identifying relevant NAM data

Enriched TxPs: Unclassified chemicals, TxP model predicted specific-acting

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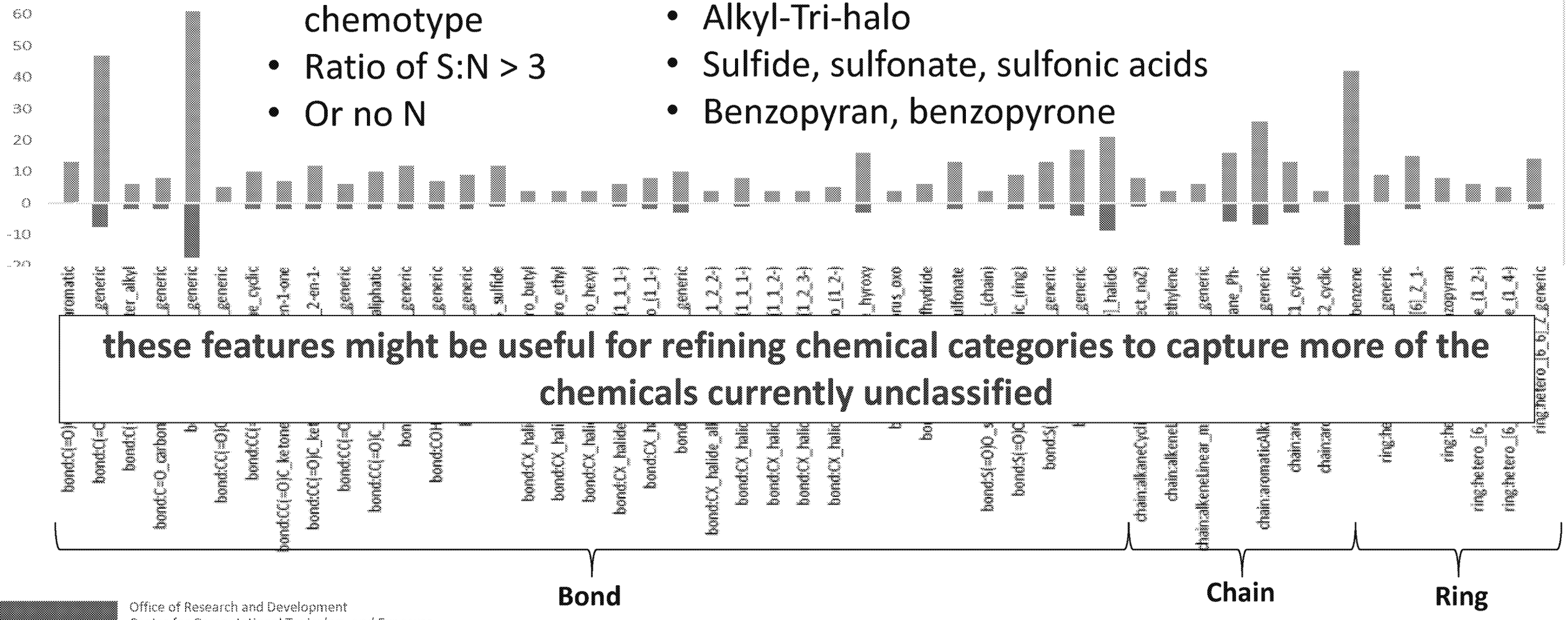
Criteria:

- ≥ 3 chemicals per chemotype
- Ratio of S:N > 3
- Or no N

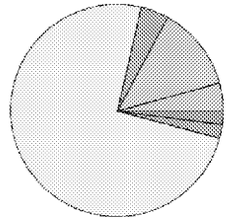
Results:

- Ketones
- Alkyl-Tri-halo
- Sulfide, sulfonate, sulfonic acids
- Benzopyran, benzopyrone



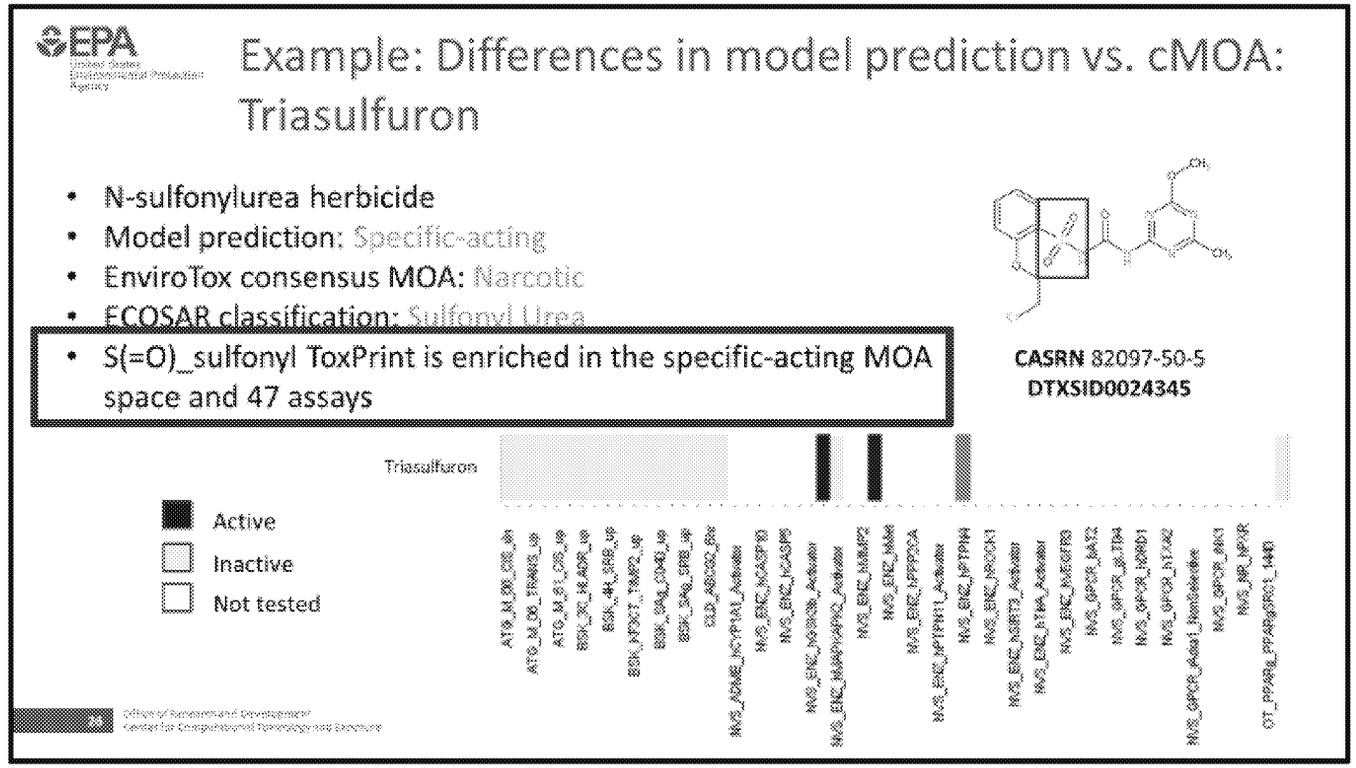
Exploring assay platforms across TxP model predicted classes

- Use chemotype enrichments to inform potential NAM data streams
- Example: sulfonyl TxP enrichments across NovaScreen (NVS) assay platform
- Identified 47 assays due to sulfonyl TxP enrichment

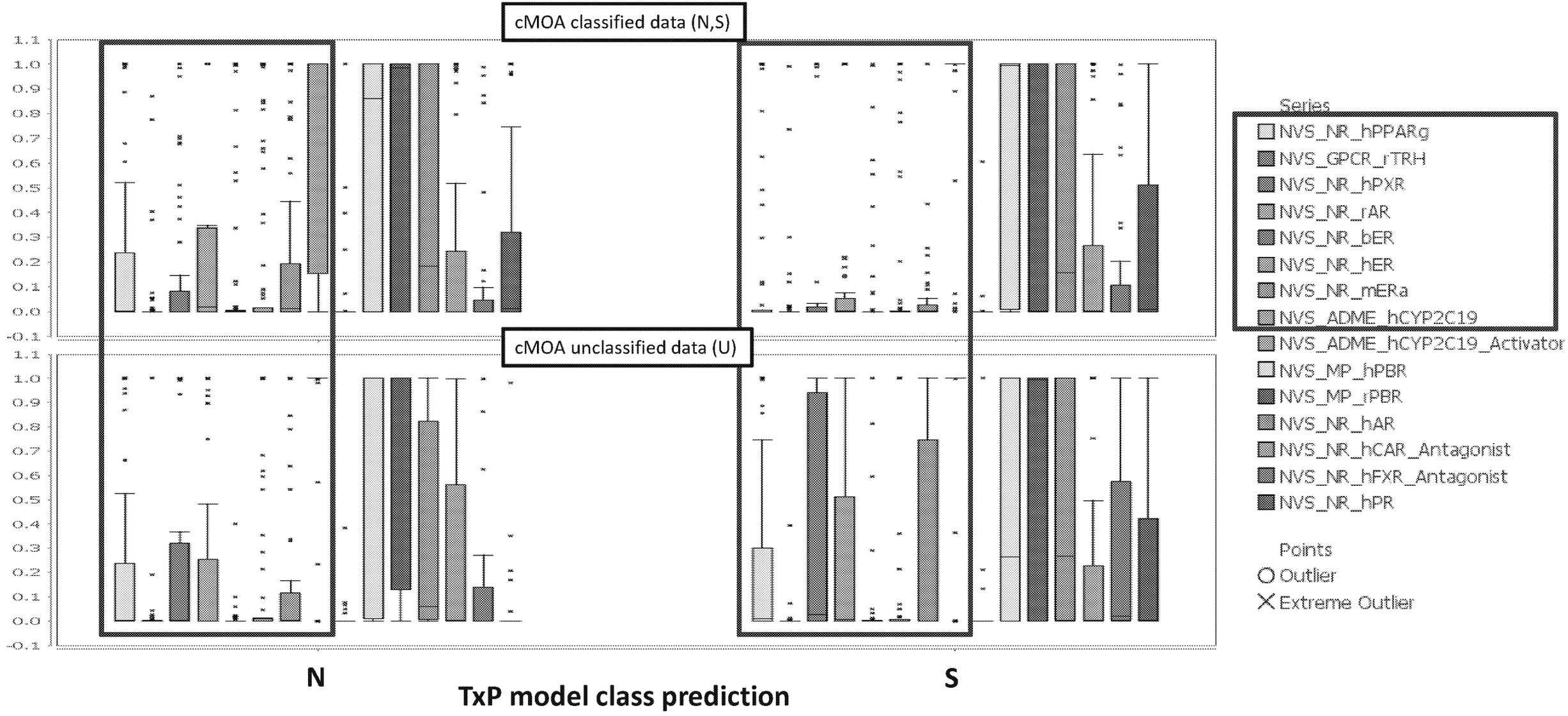


Assay platform identification:

ATG BSK CLD NVS OT TOX21



NVS Platform: TxP model class predictions



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Summary

- Increased the available chemical space of EnviroTox w/ cMOA classifications
- Developed a robust structural TxP model
  - Robust N/S classification
  - Challenges in unclassified chemistries
- Investigated model predictions to inform ECOSAR preliminary set of unclassified chemicals
  - Majority of unclassified chemicals predicted to have a specific acting MOA
  - Identified primary chemotypes for specific-acting MOAs
- Exploring methods to fold in NAM data streams
  - Using chemotype enrichments to identify potential bioassays with bioactivity to provide support of NAM data in category development

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Thank you!